

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, wherein the flow channel comprises:

a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and

a nucleic acid synthetase immobilized in the regeneration region,

wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 2 (Currently Amended): The nucleic acid amplifier of claim 1, wherein the nucleic acid amplifier amplifier further comprises a means for controlling temperature, wherein the means for controlling temperature is capable of heating the denaturation region and of keeping a temperature of the regeneration region lower than a temperature of the denaturation region.

Claim 3 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 4 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 5 (Currently Amended): The nucleic acid amplifier of claim 1, wherein the flow channel comprises at least one unit comprising the denaturation region and followed by the regeneration region alternately.

Claim 6: (Cancelled)

Claim 7 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 8 (Previously Presented): The nucleic acid amplifier of claim 1, further comprising a solution-sending device for directionally regulating a flow of the reaction solution, wherein the solution-sending device is controllable to periodically reverse the direction of flow of the reaction solution.

Claim 9 (Currently Amended): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising:

(a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;

(b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the nucleic acid synthetase has an optimum temperature of 30 to 40 °C.

Claim 10 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 11 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 12 (Currently Amended): The nucleic acid amplifier of claim 2, wherein the flow channel comprises at least one unit comprising the denaturation region followed by and the regeneration region alternately.

Claim 13 (Currently Amended): The nucleic acid amplifier of claim 3, wherein the flow channel comprises passes at least once the denaturation region and followed by the regeneration region alternately.

Claim 14 (Currently Amended): The nucleic acid amplifier of claim 4, wherein the flow channel comprises at least one unit comprising the denaturation region followed by and the regeneration region alternately.

Claims 15-18 (Cancelled)

Claim 19 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 20 (Previously Presented): The nucleic acid amplifier of claim 3, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 21 (New): The nucleic acid amplifier of claim 1, wherein the flow channel passes each of the regeneration region and the denaturation region from 20 to 40 times.

Claim 22 (New): The nucleic acid amplifier of claim 21, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least each regeneration region.

Claim 23 (New): The nucleic acid amplifier of claim 3, wherein two or more different nucleic acid synthetases are immobilized on the beads.

Claim 24 (New): The nucleic acid amplifier of claim 4, wherein two or more different nucleic acid synthetases are immobilized on the inner wall of the regeneration region.

Claim 25 (New): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized on an inner wall of the flow channel along the entire length of said flow channel.

Claim 26 (New): The nucleic acid amplifier of claim 1, wherein the flow channel comprises a semi-permeable capillary, wherein a medium of a thermostatic chamber, on which the capillary is mounted, is a reaction substrate comprising the phosphate compound and the metal ion to supply the reaction substrate continuously into the capillary.

Claim 27 (New): The nucleic acid amplifier of claim 22, wherein a portion of the denaturation region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 12 mm, wherein a portion of the regeneration region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm, and wherein a portion of the bead-filling part in one flow channel unit has the width of 1000  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm.

Claim 28 (New): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, wherein the flow channel comprises:

a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and a nucleic acid synthetase immobilized in the regeneration region, wherein the regeneration region has an optimum temperature of 30 to 40°C.

Claim 29 (New): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising:

(a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;

(b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the region in which the regeneration is performed has an optimum temperature of 30 to 40 °C.

Claim 30 (New): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid

primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, *e* wherein the flow channel comprises:

    a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

    a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and

    a nucleic acid synthetase immobilized in the regeneration region,  
    wherein the ratio in volume between the regeneration region and the denaturation region is about 7:1.

Claim 31 (New): The nucleic acid amplifier of claim 30, wherein the nucleic acid amplifier further comprises a means for controlling temperature, wherein the means for controlling temperature is capable of heating the denaturation region and of keeping a temperature of the regeneration region lower than a temperature of the denaturation region.

Claim 32 (New): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 33 (New): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 34 (New): The nucleic acid amplifier of claim 30, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 35 (New): The nucleic acid amplifier according to claim 30, wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 36 (New): The nucleic acid amplifier of claim 30, wherein the flow channel comprises a circulation flow channel, the circulation flow channel comprising the regeneration region and the denaturation region.

Claim 37 (New): The nucleic acid amplifier of claim 30, further comprising a solution-sending device for directionally regulating a flow of the reaction solution, wherein the solution-sending device is controllable to periodically reverse the direction of flow of the reaction solution.

Claim 38 (New): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising

- (a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;
- (b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the ratio in volume between the regeneration region and the denaturation region is 7:1.

Claim 39 (New): The nucleic acid amplifier of claim 31, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 40 (New): The nucleic acid amplifier of claim 31, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 41 (New): The nucleic acid amplifier of claim 31, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 42 (New): The nucleic acid amplifier of claim 32, wherein the flow channel passes at least once the denaturation region followed by the regeneration region.

Claim 43 (New): The nucleic acid amplifier of claim 33, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 44 (New): The nucleic acid amplifier according to claim 31, wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 45 (New): The nucleic acid amplifier according to claim 32, wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 46 (New): The nucleic acid amplifier according to claim 33, wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 47 (New): The nucleic acid amplifier according to claim 34, wherein the nucleic acid synthetase has an optimum temperature of 30 to 40°C.

Claim 48 (New): The nucleic acid amplifier of claim 31, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 49 (New): The nucleic acid amplifier of claim 32, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 50 (New): The nucleic acid amplifier of claim 30, wherein the flow channel passes each of the regeneration region and the denaturation region from 20 to 40 times.

Claim 51 (New): The nucleic acid amplifier of claim 50, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least each regeneration region.

Claim 52 (New): The nucleic acid amplifier of claim 32, wherein two or more different nucleic acid synthetases are immobilized on the beads.

Claim 53 (New): The nucleic acid amplifier of claim 33, wherein two or more different nucleic acid synthetases are immobilized on the inner wall of the regeneration region.

Claim 54 (New): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized on an inner wall of the flow channel along the entire length of said flow channel.

Claim 55 (New): The nucleic acid amplifier of claim 30, wherein the flow channel comprises a semi-permeable capillary, wherein a medium of a thermostatic chamber, on which the capillary is mounted, is a reaction substrate comprising the phosphate compound and the metal ion to supply the reaction substrate continuously into the capillary.

Claim 56 (New): The nucleic acid amplifier of claim 51, wherein a portion of the denaturation region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 12 mm, wherein a portion of the regeneration region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm, and wherein a portion of the bead-filling part in one flow channel unit has the width of 1000  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm.